HATCHING KIT FOR TOXICITY TEST

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 10/454,821, filed Jun. 5, 2003, which is a continuation of U.S. patent application Ser. No. 09/930,499, filed Aug. 16, 2001, which claims the benefit of priority under 35 U.S.C. §119(e) of U.S. provisional Application Ser. No. 60/225,788, filed Aug. 17, 2001, all of which are hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

Not Applicable

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the field of animal husbandry. In particular, a rapid hatching fish embryo kit and method for using same is disclosed. The invention can be used in any application where it is desirable to store and/or transport fish embryos that can be removed from the storage media and hatched, preferably within about 48 hours. For example, the present invention can be used to perform rapid toxicity assessments in the field where it would otherwise be difficult to culture the fish necessary to perform such tests. In general, the present invention can be used as a convenient means for distributing fish to investigators, aquaculturists, hobbyists, and the like.

2. Description of Related Art

Annual fish are unique in that they are common only in habitats subject to erratic climatic conditions and complete seasonal drying. As their habitats dry, all hatched fish die, and species survival is totally dependent on the adults' substrate deposition of drought-resistant eggs which are capable of entering successive stages of diapause (suspended development). These eggs remain in a state of diapause until hatching is triggered by the onset of rainfall.

The use of fish to evaluate acute toxicity in the aquatic environment is common practice. Most fish models used in standard acute toxicity testing require labor intensive culture up to the moment they are to be used in a toxicity test. Substantial amounts of time and resources are required for culturing and maintaining healthy fish, for monitoring and maintaining water quality, for monitoring and maintaining environmental conditions, and for monitoring and maintaining appropriate biological cycles. Further, existing fish models typically require critical timing, certain well developed space requirements, and specific age requirements.

Classically, the use of fish and aquatic macro-invertebrates in standard acute toxicity tests for environmental risk hazard assessments has been exceedingly labor intensive and costly because of the need to maintain continuous cultures of healthy test organisms. Consequently, as the need for more wide-spread monitoring of environmental waters and effluents for preliminary indications of toxicity became increasingly apparent, a number of alternative tests systems not requiring continuous maintenance of organisms or cultures have been developed. Termed rapid screening toxicity tests, these tests systems commonly measure bacterial bioluminescence or respiration (e.g., Microtox, Azur Environmental, Carlsbad, Calif., USA; Polytox, Bethlehem, Pa., USA) and

mortality or growth of organisms after emergence from resting life stages (e.g., rotifer cysts, brine shrimp cysts, or lettuce seeds).

In total, the existing acute toxicity testing methods involving fish present problems in terms of cost, timeliness, intensive labor requirements, and. specific environmental monitoring and maintenance.

Nothobranchius guentheri is an annual killifish indigenous to the coastal lowlands of Tanzania and Kenya. The embryonic development of *N. guentheri* has been extensively studied and is known to proceed through three successive stages, designated as diapause 1, 2, and 3. In diapause 1, the embryo is typically characterized as an undifferentiated mass of cells. In diapause 2, the embryo is typically characterized as being partially developed and having undifferentiated cells. In diapause 3, the embryo is typically characterized as being fully developed and ready to hatch in the presence of water or at the onset of rainfall.

Of the many variables affecting embryonic development, temperature has been most amply documented, and it is concluded that fully developed embryos were most sensitive to extremes and that diapause 2 embryos were less sensitive than diapause 1 embryos. Seasonal variations, including but not limited to light, light cycle, moisture content, and embryo proximity were also found to be important. Other factors known to affect the onset and duration of diapause included exposure to light, oxygen tension, partial desiccation, and exposure to ammonia.

SUMMARY OF THE INVENTION

The unique nature of the reproductive cycle of the annual killifish allows the long-term storage of diapause embryos under semi-dry laboratory conditions. Thus, toxicity evaluations are possible using fish as a test organism without the need to maintain a continuous population.

A test method has been developed in part to exploit these unique features, using newly hatched killifish (*Nothobranchius guentheri*) for rapid acute toxicity screening. Embryo culturing methods have been established and a storage media has been developed to allow convenient recovery of embryos for testing from storage media. After recovery, the embryos may be hatched from storage for use in a toxicity test.

The rapid fish acute toxicity test according to the present invention uses the killifish *Nothobranchius guentheri*. Recent studies have demonstrated that killifish can be mass cultured to produce large numbers of embryos in a suspended state (diapause). The embryos in diapause can be stored for long periods of time in semi-dry laboratory conditions for future toxicity testing. By changing the killifish embryo holding conditions, the embryos will hatch and the fry can be grown and used for testing. A fish toxicity test system that requires no culture at the time of testing would be especially useful and cost effective. The rapid killifish test could be conducted at any time without concern for culture system requirements to provide fish for testing, nor would conducting toxicity testing be subject to the vagaries of seasonal growth patterns and the delayed time associated with producing suitable test subjects.

The processes of the present invention were compared to the alternative test systems (using five representative protocols) and to standard acute toxicity tests, all of which were briefly identified in the Background section. The results for Microtox, rotifer (*Branchionus calyiflorus*), and lettuce (*Lactuca sativa*) compared favorably in sensitivity and reproducibility with those of the standard tests and were of shorter